

U.S. Serial No. 10/759,519
Amendment responsive to 4/12/2006 Office Action
Amendment dated October 12, 2006

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Amendments to the Claims:

This listing of claims will replace all prior version, and listings, of claims in the application:

Listing of the Claims:

1. (Currently Amended) A method for determining a haplotype of a subject comprising the steps of:
 - (a) diluting a nucleic acid sample from the subject into a single molecule dilution;
 - (b) amplifying the diluted single ~~nucleotide~~ molecule dilution and in a multiplex amplification reaction with at least two different primer pairs designed to amplify at least two nucleic acid regions comprising at least two polymorphic sites in the nucleic acid template;
 - (c) genotyping the at least two nucleic acid regions that contain at least one polymorphic site[[s]] in the single nucleic acid molecule; and
 - (d) determining the haplotype from the genotypes of the at least the two polymorphic sites to obtain a haplotype for the subject.
2. (Original) The method of claim 1, further comprising repeating steps a-c at least three times from the same nucleic acid sample to obtain at least four genotype replicas from the same subject and thereafter comparing the at least four genotype replicas to determine the haplotype.
3. (Original) The method of claim 2, further comprising comparing the haplotype with a haplotype from a control or a database of haplotypes from controls to determine association of the haplotype with a biological trait.
4. (Currently Amended) The method of claim 1, wherein the at least two polymorphic sites contain a polymorphism that is a single nucleotide polymorphism.
5. (Original) The method of claim 1, wherein the polymorphism is a deletion, an insertion, a substitution or an inversion.

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6. (Currently Amended) The method of claim 1, wherein the at least two polymorphic sites contain a polymorphism that is a combination of one or more markers selected from the group consisting of a single nucleotide polymorphism, deletion, an insertion, a substitution or an inversion.
7. (Currently Amended) The method of claim[[s]] 1[[-6]], wherein genotyping is performed using primer extension and mass spectrometric detection.
8. (Original) The method of claim 2, wherein 12-18 genotype replicas are produced.
9. (Currently Amended) A method of diagnosing a disease condition or disease susceptibility by determining a disease related haplotype in a subject comprising the steps of:
 - (a) diluting a nucleic acid sample from the subject into a single molecule dilution;
 - (b) amplifying the diluted single ~~nucleotide~~ molecule dilution and in a multiplex amplification reaction with at least two primer pairs designed to amplify a region comprising at least two polymorphic sites in the nucleic acid template;
 - (c) genotyping the polymorphic sites in the single nucleic acid molecule;
 - (d) determining the haplotype from the genotype of at least two polymorphic sites to obtain a haplotype for the subject; and
 - (e) comparing the haplotype of the subject to known disease-associated haplotypes, wherein a match in the sample haplotype with a disease-associated haplotype indicates that the subject has the disease or that the subject is susceptible for the disease.
10. (Original) The method of claim 9, further comprising repeating steps a – c at least three times from the same nucleic acid sample to obtain at least four genotype replicas from the same subject and thereafter comparing the at least four genotype replicas to determine the haplotype.
11. (Original) The method of claim 10, wherein 12-18 replicas are produced.

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12. (Currently Amended) A method of determining a haplotype of a subject comprising the steps of:

- (a) treating a nucleic acid sample from the subject with a composition that differentially affects an epigenetically modified nucleotide in the nucleic acid sample to effectively create polymorphisms based on the epigenetic modification;
- (b) diluting the treated nucleic acid sample into a single copy dilution;
- (c) amplifying the diluted nucleic acid sample using at least two different primer pairs in a multiplex amplification reaction;
- (d) genotyping the amplified sample; and
- (e) determining the haplotype of the subject from the genotyped sample.

13. (Original) The method of claim 12, further comprising repeating the steps b-d at least three times to obtain at least four genotype replicas from the same subject and thereafter determining a haplotype of the subject based on the genotype replicas.

14. (Original) The method of claim 13, wherein 12-18 replicas are produced.

15. (Original) The method of claim 12, wherein the epigenetically modified nucleotide is a methylated nucleotide.

16. (Original) The method of claim 15, wherein the nucleic acid sample is treated with bisulfite.

17. (Currently Amended) A method of determining a haplotype in a subject comprising the steps of:

- (a) digesting a nucleic acid sample from the subject with a methylation-sensitive restriction enzyme so that either unmethylated DNA or methylated DNA is left intact, depending on which enzyme is used;
- (b) diluting the digested nucleic acid sample to a single molecule concentration;

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(c) amplifying the diluted and undiluted nucleic acid sample with at least two different primer pairs in a multiplex amplification reaction;

(d) genotyping the amplified samples; and

(e) determining a haplotype of a methylated nucleic acid wherein at least one polymorphic marker[[s]] next to the methylation site, together with the methylation site, constitutes a haplotype.

18. (Original) The method of claim 17, further comprising repeating the steps b-d at least three times to obtain at least four genotype replicas from the same subject and thereafter determining a haplotype of the subject based on the genotype replicas.

19. (New) The method of claim 1, wherein the multiplex amplification reaction has at least 4 different primer pairs designed to amplify at least four nucleic acid regions.